

Europäisches Patentamt  
European Patent Office  
Office européen des brevets



(11) EP 0 911 396 A3

(12) EUROPEAN PATENT APPLICATION

(88) Date of publication A3:  
06.05.1999 Bulletin 1999/18

(43) Date of publication A2:  
28.04.1999 Bulletin 1999/17

(21) Application number: 98122014.8

(22) Date of filing: 02.12.1996

(51) Int Cl.<sup>6</sup>: C12N 15/10, C12N 15/64,  
C12Q 1/68  
// C07K14/435, C07K14/545,  
C12N9/86, C12N9/38,  
C12N15/52

(84) Designated Contracting States:  
AT BE CH DE DK ES FI FR GB GR IE IT LI LU MC  
NL PT SE

(30) Priority: 30.11.1995 US 564955  
25.03.1996 US 621859

(62) Document number(s) of the earlier application(s) in  
accordance with Art. 76 EPC:  
96940934.1 / 0 876 509

(71) Applicant: Maxisgen, Inc.  
Santa Clara, CA 95051 (US)

(72) Inventors:  
• Stemmer, Willem P.C.  
Los Gatos, CA 95030 (US)  
• Cramer, Andreas  
Mountain View, CA 94040 (US)

(74) Representative: Irvine, Jonquil Claire  
J.A. KEMP & CO.  
14 South Square  
Gray's Inn  
London WC1R 5LX (GB)

(54) Methods for generating polynucleotides having desired characteristics by iterative selective and recombination

(57) The present invention relates to a method for evolving a polynucleotide encoding a plurality of genes, e.g. multiple genes forming a multicomponent pathway. The method involves shuffling of polynucleotides by conducting a polynucleotide amplification process on overlapping segments of a population of variants of a polynucleotide encoding a plurality of genes under conditions whereby one segment serves as a template for extension of another segment to generate a population of recombinant polynucleotides. This population is screened for a recombinant polynucleotide encoding a plurality of genes having a desired property.

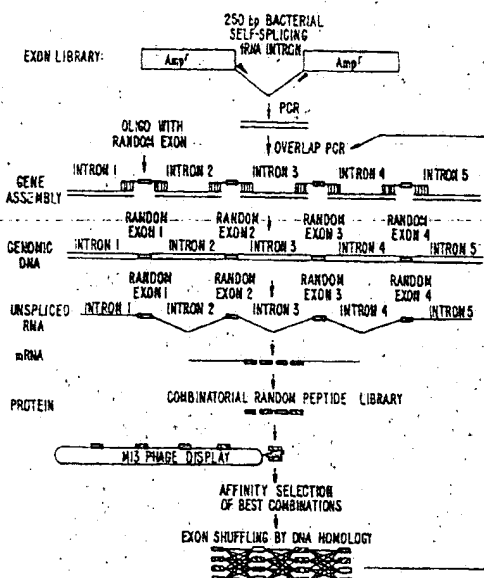


FIG. 20.

EP 0 911 396 A3



European Patent  
Office

# EUROPEAN SEARCH REPORT

Application Number  
EP 98 12 2014

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
Y	WO 95 22625 A (AFFYMAX TECH NV ;STEMMER WILLEM P C (US); CRAMERI ANDREAS (US)) 24 August 1995 * the whole document *	1-20	C12N15/10 C12N15/64 C1201/68 //C07K14/435, C07K14/545, C12N9/86, C12N9/38, C12N15/52
Y	STEMMER W: "DNA shuffling by random fragmentatio and reassembly: In vitro recombination for molecular evolution" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 91, October 1994, pages 10747-10751. XP002087463 * the whole document *	1-20	
Y	STEMMER W P C: "Rapid evolution of a protein in vitro by DNA shuffling" NATURE, vol. 370, 4 August 1994, pages 389-391. XP002082182 * the whole document *	1-20	
Y	W.P.C. STEMMER: "Searching sequence space" NATURE BIOTECHNOLOGY, vol. 13, June 1995, pages 549-553. XP002095510 NATURE PUBL. CO., NEW YORK, US * the whole document *	1-20	TECHNICAL FIELDS SEARCHED (Int.Cl.6) C12N C120
Y	US 5 279 952 A (WU KUN C) 18 January 1994 * the whole document *	1-20	
Y	US 5 223 408 A (GOEDEL DAVID V ET AL) 29 June 1993 * the whole document *	1-20	
-/-			
The present search report has been drawn up for all claims			
Place of search <b>THE HAGUE</b>		Date of completion of the search <b>4 March 1999</b>	Examiner <b>Hornig, H</b>
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document			

EPO FORM 1503 03/82 (Pdc/Cl)



European Patent  
Office

## EUROPEAN SEARCH REPORT

Application Number  
EP 98 12 2014

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
Y	R M HORTON ET AL: "Engineering hybrid genes without the use of restriction enzymes: gene splicing by overlap extension" GENE, vol. 77, 1989, pages 61-68, XP002090392 * the whole document *	1-20	TECHNICAL FIELDS SEARCHED (Int.Cl.6)
Y	HO S N ET AL: "SITE-DIRECTED MUTAGENESIS BY OVERLAP EXTENSION USING THE POLYMERASE CHAIN REACTION" GENE, vol. 77, no. 1, 1 January 1989, pages 51-59, XP000272761 * the whole document *	1-20	
A	WO 91 07506 A (US GOVERNMENT) 30 May 1991 * the whole document *	1-20	
A	HEIM R ET AL: "WAVELENGTH MUTATIONS AND POSTTRANSLATIONAL AUTOXIDATION OF GREEN FLUORESCENT PROTEIN" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 91, 1 December 1994, pages 12501-12504, XP000574454 * the whole document *	1-20	
P,X	A. CRAMERI ET AL.: "Improved green fluorescent protein by molecular evolution using DNA shuffling" NATURE GENETICS, vol. 14, March 1996, pages 315-319, XP002095449 NATURE PUBLISHING CO., NEW YORK, US * the whole document *	1-16	
-/--			
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 4 March 1999	Examiner Hornig, H
CATEGORY OF CITED DOCUMENTS		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document	
X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document			

EPO FORM 1503 (03.92) (P4/C01)



European Patent  
Office

## EUROPEAN SEARCH REPORT

Application Number

EP 98 12 2014

## DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
L	WO 97 35966 A (MAXYGEN INC ; MINSHULL JEREMY (US); STEMMER WILLEM P C (US)) 2 October 1997 * examples I-IV. *	1-16	
T	CRAMER A ET AL: "Molecular evolution of an arsenate detoxification pathway y DNA shuffling" NATURE BIOTECHNOLOGY, vol. 15, May 1997, pages 436-438. XP002082183 * the whole document *	1-16	
			TECHNICAL FIELDS SEARCHED (Int.Cl.6)
The present search report has been drawn up for all claims			

Place of search

THE HAGUE

Date of completion of the search

4 March 1999

Examiner

Hornig, H

## CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone  
 Y : particularly relevant if combined with another document of the same category  
 A : technological background  
 O : non-written disclosure  
 P : intermediate document

T : theory or principle underlying the invention  
 E : earlier patent document, but published on, or after the filing date  
 D : document cited in the application  
 L : document cited for other reasons

& : member of the same patent family, corresponding document

**ANNEX TO THE EUROPEAN SEARCH REPORT  
ON EUROPEAN PATENT APPLICATION NO.**

EP 98 12 2014

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report.  
The members are as contained in the European Patent Office EDP file on  
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

04-03-1999

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9522625 A	24-08-1995	US 5605793 A	25-02-1997
		AU 2971495 A	04-09-1995
		CA 2182393 A	24-08-1995
		CN 1145641 A	19-03-1997
		EP 0752008 A	08-01-1997
		JP 10500561 T	20-01-1998
		US 5811238 A	22-09-1998
		US 5837458 A	17-11-1998
US 5279952 A	18-01-1994	NONE	
US 5223408 A	29-06-1993	US 5736135 A	07-04-1998
WO 9107506 A	30-05-1991	AU 6886991 A	13-06-1991
WO 9735966 A	02-10-1997	US 5837458 A	17-11-1998
		AU 2337797 A	17-10-1997
		AU 2542697 A	17-10-1997
		WO 9735957 A	02-10-1998
		AU 1087397 A	19-06-1997
		CA 2239099 A	05-06-1997
		EP 0876509 A	11-11-1998
		WO 9720078 A	05-06-1997

EPO FORM P0469

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82